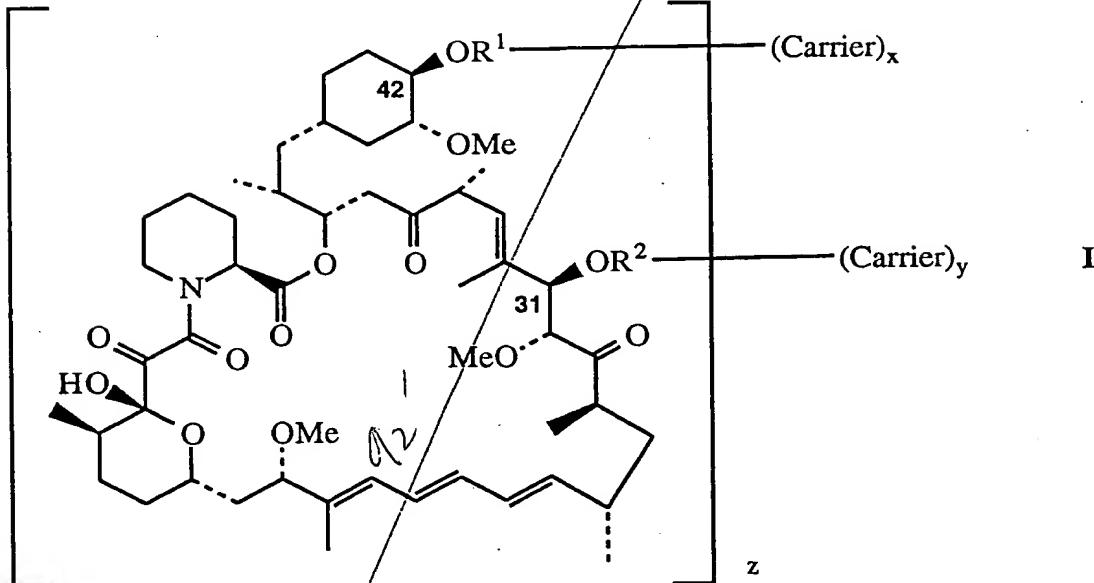


CLAIMS

What is claimed is:

1. A rapamycin conjugate of formula I, having the structure

5



I

wherein R<sup>1</sup> and R<sup>2</sup> are each, independently, hydrogen or -(R<sup>3</sup>-L-R<sup>4</sup>)<sub>a</sub>-;

L is a linking group;

10 R<sup>3</sup> is selected from the group consisting of carbonyl, -S(O)-, -S(O)<sub>2</sub>, -P(O)<sub>2</sub>-,  
-P(O)(CH<sub>3</sub>)-, -C(S)-, and -CH<sub>2</sub>C(O)-;

R<sup>4</sup> is selected from the group consisting of carbonyl, -NH-, -S-, -CH<sub>2</sub>-, and -O-;

a = 1 - 5;

x = 0 - 1;

15 y = 0 - 1;

z is from about 1 to about 120;

and Carrier is immunogenic carrier material, detector carrier material, or a solid matrix,  
or a salt thereof with the proviso that R<sup>1</sup> and R<sup>2</sup> are both not hydrogen; and further  
provided that when a is greater than 1, each L group can be the same or different; and  
20 still further provided that x is 0 if R<sup>1</sup> is hydrogen and y is 0 if R<sup>2</sup> is hydrogen, and if x  
and y are both 1, the Carrier moiety is the same in both cases.

2. An antibody, capable of specifically binding with rapamycin prepared against a  
conjugate of claim 1.

3. In an immunoassay method for determining levels of rapamycin or a derivative thereof, the improvement comprises employing an antibody prepared against a conjugate of claim 1.

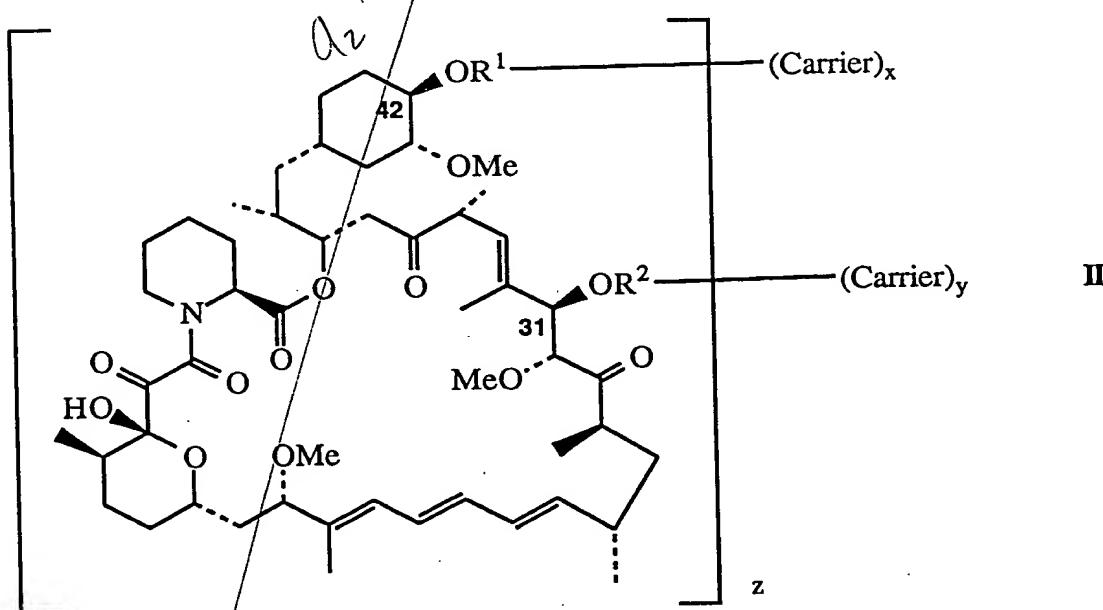
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4. In an immunoassay method for determining levels of rapamycin or a derivative thereof, the improvement comprises using a conjugate of claim 1 as a detector molecule.

10 5. A test kit for measuring levels of rapamycin or a derivative thereof comprising a rapamycin conjugate of claim 1 bound to a solid support and an antibody capable of specifically binding to rapamycin.

15 6. A rapamycin conjugate of formula II, having the structure

15



R¹ and R² are each, independently, hydrogen or -R³-L-R⁴- ;

L is -A-(CR⁵R⁶)ₘ[B-(CR⁷R⁸)ₙ]e- ;

A is -CH₂- or -NR⁹- ;

20 B is -O- , -NR⁹- , -S- , -S(O)- , or -S(O)₂- ;

R³ is selected from the group consisting of carbonyl, -S(O)- , -S(O)₂ , -P(O)₂- , -P(O)(CH₃)- , -C(S)- , and -CH₂C(O)- ;

R⁴ is selected from the group consisting of carbonyl, -NH- , -S- , -CH₂- , and -O- ;

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R<sup>5</sup>, R<sup>6</sup>, R<sup>7</sup>, and R<sup>8</sup> are each, independently, hydrogen, alkyl of 1-6 carbon atoms, alkenyl of 2-7 carbon atoms, alkynyl of 2-7 carbon atoms, halo, hydroxy, trifluoromethyl, arylalkyl of 7-10 carbon atoms, aminoalkyl of 1-6 carbon atoms, hydroxyalkyl of 1-4 carbon atoms, alkoxy of 1-6 carbon atoms, carbalkoxy of 2-7 carbon atoms, cyano, amino, -CO<sub>2</sub>H, or phenyl which is optionally mono-, di-, or tri-substituted with a substituent selected from alkyl of 1-6 carbon atoms, alkoxy of 1-6 carbon atoms, hydroxy, cyano, halo, nitro, carbalkoxy of 2-7 carbon atoms, trifluoromethyl, amino, or -CO<sub>2</sub>H;

R<sup>9</sup> is hydrogen, alkyl of 1-6 carbon atoms, or aralkyl of 7-10 carbon atoms;

10 b = 0-10;

d = 0-10;

e = 0-2;

x = 0 - 1;

y = 0 - 1;

15 z is from about 1 to about 120;

and Carrier is immunogenic carrier material, detector carrier material, or a solid matrix, or a salt thereof with the proviso that R<sup>1</sup> and R<sup>2</sup> are both not hydrogen; and further provided that when b is greater than 1, each of the CR<sup>5</sup>R<sup>6</sup> groups can be the same or different, and when d is greater than 1, each of the CR<sup>7</sup>R<sup>8</sup> groups can be the same or different; and still further provided that x is 0 if R<sup>1</sup> is hydrogen and y is 0 if R<sup>2</sup> is hydrogen, and if x and y are both 1, the Carrier moiety is the same in both cases.

7. The conjugate of claim 6, which is rapamycin 42-ester with succinic acid conjugate with keyhole limpet hemocyanin.

25

8. The conjugate of claim 6, which is rapamycin 42-ester with succinic acid conjugate with ovalbumin.

30

9. The conjugate of claim 6, which is rapamycin 42-ester with succinic acid conjugate with horseradish peroxidase.

10. The conjugate of claim 6, which is rapamycin 31,42-diester with glutaric acid conjugate with keyhole limpet hemocyanin.

35

11. The conjugate of claim 6, which is rapamycin 31,42-diester with glutaric acid conjugate with horseradish peroxidase.

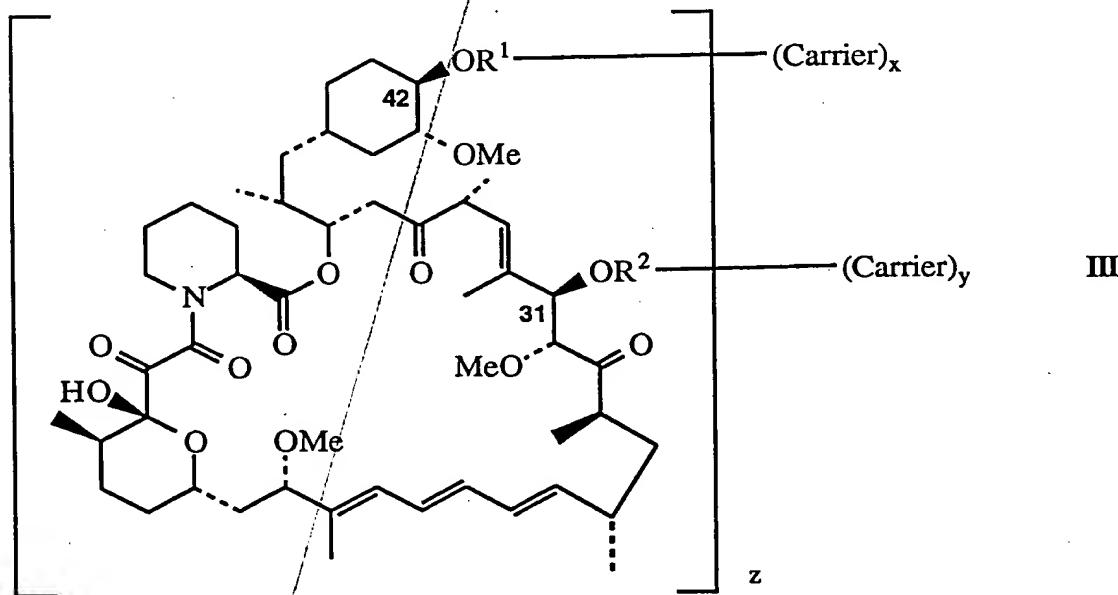
12. An antibody, capable of specifically binding with rapamycin prepared against a conjugate of claim 6.

5 13. In an immunoassay method for determining levels of rapamycin or a derivative thereof, the improvement comprises employing an antibody prepared against a conjugate of claim 6.

10 14. In an immunoassay method for determining levels of rapamycin or a derivative thereof, the improvement comprises using a conjugate of claim 6 as a detector molecule.

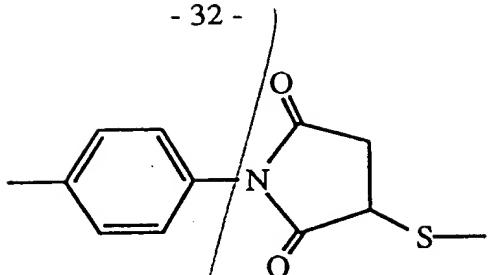
15 15. A test kit for measuring levels of rapamycin or a derivative thereof comprising a rapamycin conjugate of claim 6 bound to a solid support and an antibody capable of specifically binding to rapamycin.

16. A rapamycin conjugate of formula III, having the structure



20 R<sup>1</sup> and R<sup>2</sup> are each, independently, hydrogen or -(R<sup>3</sup>-L<sup>1</sup>-R<sup>4</sup>)<sub>f</sub>-(R<sup>10</sup>-L<sup>2</sup>-R<sup>11</sup>)<sub>g</sub>-Carrier;  
L<sup>1</sup> is -(CH<sub>2</sub>)<sub>h</sub>-CHR<sup>12</sup>-CH<sub>2</sub>)<sub>j</sub>-;  
L<sup>2</sup> is -(CH<sub>2</sub>)<sub>k</sub>-D-(CH<sub>2</sub>)<sub>m</sub>-E-;

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D is  $-\text{CH}_2-$ ,  $-\text{S}-\text{S}-$ , or

E is  $-\text{CH}_2-$  or  $-\text{C}(=\text{N}\text{H}_2^+\text{Cl}^-)-$  ;

R<sup>3</sup> and R<sup>10</sup> are each, independently, selected from the group consisting of carbonyl,  $-\text{S}(\text{O})-$ ,  $-\text{S}(\text{O})_2$ ,  $-\text{P}(\text{O})_2-$ ,  $-\text{P}(\text{O})(\text{CH}_3)-$ ,  $-\text{C}(\text{S})-$ , and  $-\text{CH}_2\text{C}(\text{O})-$  ;

5 R<sup>4</sup> and R<sup>11</sup> are each, independently, selected from the group consisting of carbonyl,  $-\text{NH}-$ ,  $-\text{S}-$ ,  $-\text{CH}_2-$ , and  $-\text{O}-$  ;

10 R<sup>12</sup> is hydrogen, alkyl of 1-6 carbon atoms, arylalkyl of 7-10 carbon atoms, alkenyl of 2-7 carbon atoms, alkynyl of 2-7 carbon atoms,  $-(\text{CH}_2)_n\text{CO}_2\text{R}^{13}$ ,  $-(\text{CH}_2)_p\text{NR}^{14}\text{R}^{15}$ , carbamylalkyl of 2-3 carbon atoms, aminoalkyl of 1-4 carbon atoms, hydroxyalkyl of 1-4 carbon atoms, guanylalkyl of 2-4 carbon atoms, mercaptoalkyl of 1-4 carbon atoms, alkylthioalkyl of 2-6 carbon atoms, indolylmethyl, hydroxyphenylmethyl, imidazoylmethyl, halo, trifluoromethyl, or phenyl which is optionally mono-, di-, or tri-substituted with a substituent selected from alkyl of 1-6 carbon atoms, alkoxy of 1-6 carbon atoms, hydroxy, cyano, halo, nitro, carbalkoxy of 2-7 carbon atoms, trifluoromethyl, amino, or  $-\text{CO}_2\text{H}$ ;

15 R<sup>14</sup>, and R<sup>15</sup> are each, independently, hydrogen, alkyl of 1-6 carbon atoms, or arylalkyl of 7-10 carbon atoms;

20 R<sup>13</sup> is hydrogen, alkyl of 1-6 carbon atoms, arylalkyl of 7-10 carbon atoms, alkenyl of 2-7 carbon atoms, alkynyl of 2-7 carbon atoms, or phenyl which is optionally mono-, di-, or tri-substituted with a substituent selected from alkyl of 1-6 carbon atoms, alkoxy of 1-6 carbon atoms, hydroxy, cyano, halo, nitro, carbalkoxy of 2-7 carbon atoms, trifluoromethyl, amino, or  $-\text{CO}_2\text{H}$ ;

f = 0-3;

25 g = 0-1;

j = 0-10;

k = 0-10;

m = 0-10;

n = 0-6;

30 p = 0-6;

x = 0 - 1;

y = 0 -1;

z is from about 1 to about 120;

and Carrier is immunogenic carrier material, detector carrier material, or a solid matrix, or a salt thereof with the proviso that R<sup>1</sup> and R<sup>2</sup> are both not hydrogen; and further provided that f and g are both not 0 and when f is greater than 1, each of the -(R<sup>3</sup>-L<sup>1</sup>-R<sup>4</sup>)- moieties can be the same or different; and still further provided that x is 0 if R<sup>1</sup> is hydrogen and y is 0 if R<sup>2</sup> is hydrogen; and if x and y are both 1, the Carrier moiety is the same in both cases.

10 17 The conjugate of claim 16, which is rapamycin 42-ester with 3-[3-(4-iminobutylthio)succinimidyl]phenylglycine conjugate with horseradish peroxidase.

15 18. The conjugate of claim 16, which is rapamycin 42 ester with (N-(3-carboxyphenyl)-3-thiosuccinimidyl)-glycine conjugate with horseradish peroxidase.

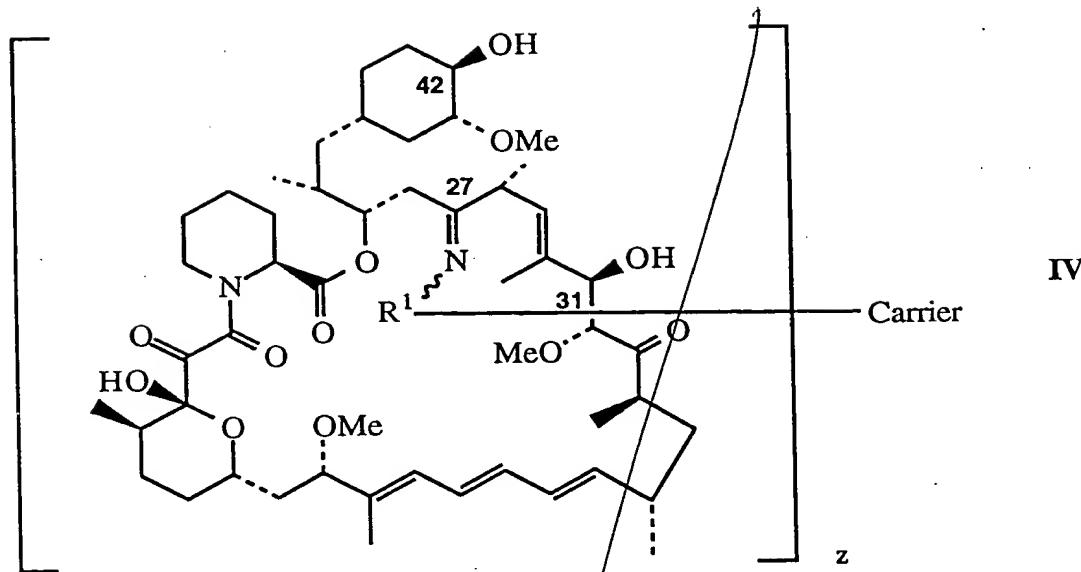
19. An antibody, capable of specifically binding with rapamycin prepared against a conjugate of claim 16.

20. In an immunoassay method for determining levels of rapamycin or a derivative thereof, the improvement comprises employing an antibody prepared against a conjugate of claim 16.

25 21. In an immunoassay method for determining levels of rapamycin or a derivative thereof, the improvement comprises using a conjugate of claim 16 as a detector molecule.

30 22. A test kit for measuring levels of rapamycin or a derivative thereof comprising a rapamycin conjugate of claim 16 bound to a solid support and an antibody capable of specifically binding to rapamycin.

23. A rapamycin conjugate of formula IV, having the structure



wherein  $R^1$  is  $-OCH_2(CH_2)_qR^{4-}$ ;

$R^4$  is selected from the group consisting of carbonyl,  $-NH-$ ,  $-S-$ ,  $-CH_2-$ , and  $-O-$ ;

$q = 0 - 6$ ;

5  $z$  is from about 1 to about 120;

and Carrier is immunogenic carrier material, detector carrier material, or a solid matrix, or a salt thereof.

24. An antibody, capable of specifically binding with rapamycin prepared against a  
10 conjugate of claim 23.

25. In an immunoassay method for determining levels of rapamycin or a derivative thereof, the improvement comprises employing an antibody prepared against a conjugate of claim 23.

15 26. In an immunoassay method for determining levels of rapamycin or a derivative thereof, the improvement comprises using a conjugate of claim 23 as a detector molecule.

20 27. A test kit for measuring levels of rapamycin or a derivative thereof comprising a rapamycin conjugate of claim 23 bound to a solid support and an antibody capable of specifically binding to rapamycin.

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28. A monoclonal antibody specifically binding to rapamycin which is designated as RAP-42-OVAF<sub>2</sub>#1MoAb.

29. A hybridoma cell line capable of producing rapamycin specific antibodies which, 5 is designated as RAP-42-OVAF<sub>2</sub>#1hc-.

30. A test kit for measuring levels of rapamycin or a derivative thereof, comprising a rapamycin specific antibody bound to a solid support.

10 31. A test kit for measuring levels of rapamycin or a derivative thereof, comprising a molecule bound to a solid support capable of capturing a rapamycin specific antibody.

15 32. The test kit according to claim 31 wherein the bound molecule is goat anti-mouse antibody.

ADD G3  
ADD B3  
ADD C2